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Metabolic control of avocado fruit growth: 3-hydroxy-3-methylglutaryl coenzyme a reductase, active oxygen species and the role of C7 sugars

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The presence of the seven carbon (7C) sugar D-*manno*-heptulose (D-*manno*-2-ketoheptose) and its corresponding sugar alcohol perseitol (D-*glycero*-D-*galacto*-heptitol) as the predominant sugars in avocado fruits is of considerable interest for two reasons. First, D-*manno*-heptulose is a potent inhibitor of plant and animal hexokinase and second, nothing is known about the role of 7C sugars in avocado fruit growth and development. In this paper the contribution of avocado 7C sugars was explored. Results show that D-*manno*-heptulose and perseitol are major soluble sugars in phloem sap, leaf petiole exudates, and in seed and mesocarp of avocado. Whereas levels of perseitol remained constant throughout fruit growth, D-*manno*-heptulose levels increased linearly until maturity. D-*manno*-heptulose does not appear to be a precursor of perseitol in fruit tissue. However, exogenous D-*manno*-heptulose increased the endogenous pool of perseitol in avocado seed and enhanced activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR, EC 1.1.1.34). Although perseitol did not affect seed HMGR when applied to whole fruit, this 7C sugar effectively negated the deleterious effects of *tert*-butylhydroperoxide and hydrogen peroxide in *in vitro* assays. It is concluded that D-*manno*-heptulose and perseitol fulfill several important functions during avocado fruit growth. These are: modulation of carbon flux, and as a protectant against reactive oxidative damage of key enzymes such as HMGR required for growth and development of the fruit.

Introduction

The biosynthesis of isoprenoids is essential for normal plant growth and development. This class of compound includes phytosterols, carotenoids, several plant hormones (abscisic acid, brassinolides; gibberellins), the side chain of cytokinin (CK), and the polyisoprenoid moiety of dolichols, quinones and regulatory proteins (Bach 1995, Chappell 1995, Rodríguez-Concepción *et al*. 1999, Galichet and Gruissem 2003). Thus, it is not surprising that several studies have demonstrated a requirement for isoprenoid synthesis in fruit growth (Cowan *et al*. 2001). For example, inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR, EC 1.1.1.34), which catalyses the NADPH-dependent formation of mevalonic acid (MVA) from 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), using statins significantly reduced the growth of tomato (Narita and Gruissem 1989, Gillaspy *et al*. 1993) and avocado fruit (Cowan *et al*. 1997). In avocado fruit, phytosterols were unable to restore growth and HMGR activity of treated fruit, whereas the CK, isopentenyladenine (2iP), negated the effect of mevastatin treatment in all phases of fruit growth and restored endogenous HMGR activity (Cowan *et al*. 1997). Furthermore, mevastatin-treated fruit contained substantially more abscisic acid (ABA) than untreated fruit and co-injection with 2iP reduced ABA to control levels. Also, treatment of growing fruit *in situ* with ABA reduced HMGR activity and fruit growth, and exacerbated fruit abscission, processes that were negated in the presence of equal amounts of 2iP (Cowan *et al*. 1997). These observations suggested that alterations in the 2iP:ABA ratio might be an important determining parameter in the control of avocado fruit growth. Support was obtained by studying the effect of 2iP and ABA on symplastic solute transport and cell-to-cell chemical communication in avocado mesocarp and seed coat tissue (Moore-Gordon *et al*. 1998). Thus, 2iP negated the deleterious effects of ABA with respect to fruit size, seed coat senescence, sucrose allocation, and membrane electrical potential and plasmodesmatal structure-function.

A more detailed study of the interaction between CK and ABA in avocado revealed that 2iP impacted ABA by promoting its oxidative catabolism (Cowan *et al*. 1999). Moreover, analysis of the response of ABA metabolism to allopurinol (an inhibitor of purine metabolism), tungstate (an inhibitor of aldehyde oxidase (AO) activity) and molybdate led to the proposal that CK-induced CK oxidase and adenine arrested activity of xanthine dehydrogenase thereby increasing the availability of molybdenum (as the molybdenum-cofactor) for sulphurylation and activation of the AOs (Taylor and Cowan 2001).

Plant AO, like its animal counterpart, uses molecular oxygen in the catalytic reaction (Koshiba *et al*. 1996). In animal systems it has been demonstrated that both superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are formed in either a one-electron or two-electron transfer involving molecular oxygen as a co-substrate for AO (Beedham 1985). Activity of plant AO, particularly during periods of enhanced ABA and/or auxin production, may also generate reactive oxygen species (ROS). ABA has been shown to influence the expression of the genes encoding superoxide dismutase isozymes (Williamson and Scandalios 1992, Zhu and Scandalios 1994, Bueno *et al*. 1998). CK too, can affect changes in the concentration of ROS. For example, CK treatment increases the concentration of ROS during pathogen infection, drought and other stresses (Hare *et al*. 1997). The increase seems to be necessary to facilitate H_2O_2-induced transcriptional activation of stress-response genes as part of the systemic acquired resistance response (Chen *et al*. 1993). This is supported by the demonstration that zeatin repressed a soybean Fe-containing superoxide dismutase gene in cells starved of CK (Crowell and Amasino 1991) while benzyladenine decreased the level of catalase mRNA transcripts in etiolated cucumber cotyledons (Toyama *et al*. 1995). Interestingly, the reaction catalysed by 2iP-oxidase yields, in addition to adenine, 3-methyl-2-butanal and H_2O_2 (Jones and Schreiber 1997). Thus, the proposed interaction between CK and ABA metabolism referred to above (Cowan *et al*. 1999, Taylor and Cowan 2001) has the potential to increase ROS production in plants.

In animal cells the oxidative interaction of O_2^- and H_2O_2 is important in the adjustment of the redox states of signal transduction proteins (e.g. growth factors, receptors, protein kinases and protein phosphatases) to ensure optimal functioning within cells. Using BHK-21 hamster fibroblasts it was shown that processes leading to either abnormally high or abnormally low levels of H_2O_2 depress rates of cell proliferation and increase apoptosis (Burdon 1996). H_2O_2 when in excess has also been shown to irreversibly inactivate animal HMGR and reduce cell division cycle activity (Omkumar and Ramasarma 1993). Since MVA is required for cell proliferation in fruit growth (Cowan *et al*. 1997, Jelesko *et al*. 1999, Mitchell and Cowan 2003) operation of a similar mechanism in plants may explain differences in fruit size within the same species and cultivar, a phenomenon typical of 'Hass' avocado.

In this paper experiments carried out to assess the potential involvement of ROS in modulating HMGR activity in avocado seed tissue are described. Additionally, since polyols are efficient scavengers of free radicals (Smirnoff and Cumbes 1989, Jennings *et al*. 1998) the role of the avocado-specific C7 sugar alcohol, D-*glycero*-D-*galacto*-heptitol (perseitol) and its reduced form, D-*manno*-heptulose, in preventing ROS-mediated irreversible inactivation of HMGR was also examined. The results indicate that HMGR activity in avocado seed tissue is sensitive to H_2O_2 and suggest that perseitol and D-*manno*-heptulose, which are related by oxidation and reduction, scavenge ROS that would otherwise inactivate HMGR during the normal course of fruit growth.

Materials and Methods

Isotopes, reactive oxygen species and C7 sugars

DL-[3-^{14}C]HMG-CoA (58.0mCi mmol^{-1}) and [U-^{14}C]sucrose (23.2Gbq mmol^{-1}) were purchased from Amersham International, Buckinghamshire, UK. Perseitol, D-*manno*-heptulose, 2iP and methyl viologen (MV) were purchased from Sigma Chemical Company, St Louis, MO, USA. Sodium azide (NaN_3) was purchased from BDH Chemicals, Poole, England and *tert*-butyl hydroperoxide (*t*-BuOOH) from Merck, Darmstadt, Germany.

Plant material and application of chemicals

Unless otherwise stated fruit were harvested 180d after full bloom (DAFB), in the early morning, and transported to the laboratory immediately. Seed tissue was dissected from the fruits, finely chopped, freeze-dried, milled into a homogeneous powder, and analysed. For studies using intact fruit, the pedicels of freshly harvested fruit were re-cut under water and the fruit stood in test tubes containing the compounds of interest, which were pulsed into the fruit under ambient conditions. Fruit were then incubated at 25°C for 24h, after which the seed was removed and the tissue prepared as described above prior to analysis.

Carbohydrate extraction and analysis

Petiole sap was obtained from freshly harvested leaves using a pressure bomb. Phloem sap was obtained from 1.0cm i.d. holes drilled equidistant about the circumference of branches to which were attached syringes for sap collection.

Water-soluble carbohydrates were extracted from sap, seed and/or mesocarp tissue either into water or 80% ethanol as previously described (Richings *et al*. 2000). Samples were resuspended in ultra-pure water, filtered through a 0.45µm nylon filter and the content and composition of carbohydrates routinely determined by HPLC analysis on a 8µm Ca-monosacharride column (300mm x 7.8mm i.d. Resex, Phenomenex, Torrance, CA, USA) with water as the mobile phase and eluted at a flow rate of 0.6ml min^{-1} and 85°C. Compounds of interest were detected using a differential refractive index detector (Waters R401, Millipore Corporation, Bedford, MA, USA) and quantified by peak integration (Data Jet integrator, Thermo Separations Products, Fremont, CA, USA) after calibration with authentic standards. Using this system, the retention time (min) of the authentic standards was: sucrose (9.1), glucose (10.9), D-*manno*-heptulose (12.5), fructose (13.4), sedoheptulose (13.5), mannitol (16.9), perseitol (19.2), and sorbitol (20.5).

HMGR extraction and assays

HMGR activity was determined using Ca^{2+}-sedimented microsomal membranes from freeze-dried seed tissue by monitoring the *in vitro* NADPH-dependent reduction of [3-^{14}C]HMG-CoA to MVA, as previously described (Cowan *et al*. 1997).

Effect of ROS and perseitol on HMGR activity

Ca^{2+}-sedimented microsomal membranes were either incubated with ROS, under standard HMGR assay conditions or were preincubated with ROS, with or without the addition of perseitol (30mM), at 30°C for 20min. HMGR activity was measured as described above. For the effect of ROS on the time course of HMGR activity, microsomes were preincubated with ROS, at 30°C for 20min and the HMGR activity assayed under standard conditions at the intervals specified in 'Results'.

Results

Sugar transport and metabolism in avocado

Analysis by reversed phase high performance liquid chromatography (HPLC) of phloem sap extracted from the trunk (mobilised carbohydrate reserves) and leaf petioles (photosynthetically produced sugars) of avocado trees revealed the composition shown in Figure 1. The major sugars included D-*manno*-heptulose (27%), perseitol (15%), and an unidentified sugar (40%) while sucrose, glucose and fructose are minor sugars usually in amounts of 5% or less of the total soluble sugar component. Thus, D-*manno*-heptulose and perseitol are mobile in the tree and available for fruit growth and development. By monitoring the change in fruit sugar content and composition over the course of development and until harvestable maturity, the fate of tree-derived sugars could be partially deduced. Figure 2 shows that in young fruit fructose was at high concentration and declined from 22% to about 4% of the total soluble sugars before harvest. Similarly, levels of glucose declined from about 19% to less than 1% of total soluble sugars. The concentration of perseitol remained fairly constant throughout avocado fruit growth whereas D-*manno*-heptulose levels rose from 15% in immature fruit to 53% of the total soluble sugars in fruit near harvestable maturity.

To gain insight into the metabolic relationship between these five major sugars fruit were pulsed with [U-^{14}C]sucrose and the distribution of radioactivity in the various sugars analysed after purification by HPLC. The bulk of the supplied [^{14}C] was associated with glucose (33%) and fructose (41%). Although only 10% of the supplied [^{14}C] remained as sucrose, no [^{14}C] was detected in the perseitol fraction whereas D-*manno*-heptulose accounted for 12% of the total radioactivity (data not shown).

Inhibition of HMGR by ROS

Treatment of freshly harvested fruit, via the pedicel, with solutions of MV, *t*-BuOOH, H_2O_2 or NaN_3 reduced endogenous HMGR activity in seed tissue of avocado (Figure 3). In response to increasing concentrations of ROS the degree of HMGR inactivation was more severe and appeared to reach completion at approximately 10mM. Since the HMGR assay mixture contained sufficient HMG-CoA, NADPH and DTT to sustain maximum activity during the 45min reaction period (Cowan *et al*. 1997), it was considered that the decrease in activity was the result of enzyme inactivation and not due to loss of substrate and/or thiol cofactor. To investigate this aspect in more detail the effect of ROS on HMGR activity *in vitro* was examined. As illustrated in Figure 4 neither MV nor NaN_3 at the concentrations tested had any significant inhibitory effect on HMGR activity. In fact, the results show that NaN_3 stimulated enzyme activity when supplied at 50mM. By comparison, whereas 1mM *t*-BuOOH did not appear to affect *in vitro* HMGR activity, addition of this ROS-potentiating compound at 10mM and 50mM resulted in a 10% and 50% reduction respectively. Surprisingly and in contrast to its effect *in vivo*, H_2O_2 did not seem to influence HMGR activity when added to the assay mixture.

H_2O_2 is thought to exert its effect on proteins indirectly through the generation of the hydroxyl radical in the presence of trace amounts of ferrous ions (Stadtman and Oliver 1991,

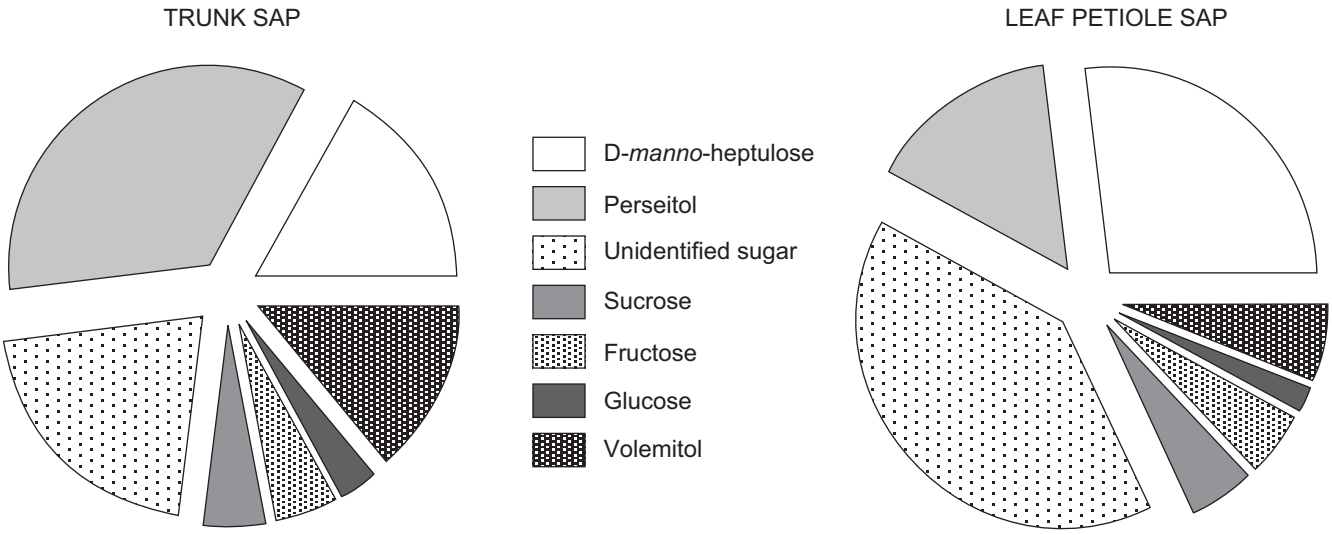

Figure 1: HPLC determination of soluble sugar content and composition of sap collected from the trunk and the leaf petioles of 'Hass' avocado trees during active fruit growth (120 DAFB). Samples were extracted and analysed as described in Materials and Methods (n = 3)

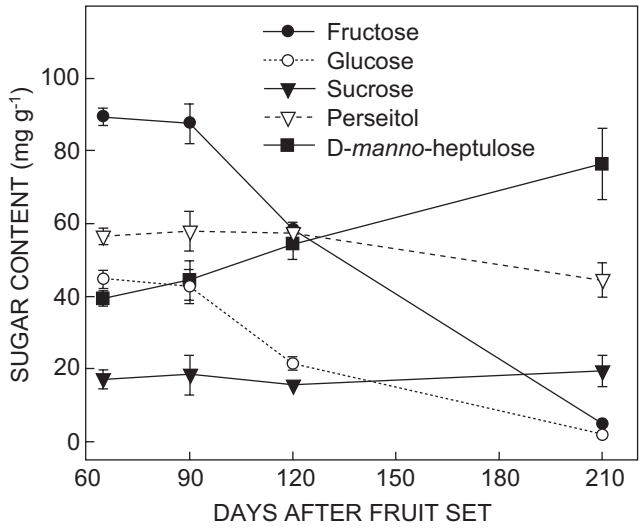

Figure 2: Changes in the soluble sugar content and composition of 'Hass' avocado fruit during the course of fruit growth and development. Samples were prepared from at least four fruit and the sugars quantified by HPLC. Data are the mean ± SE

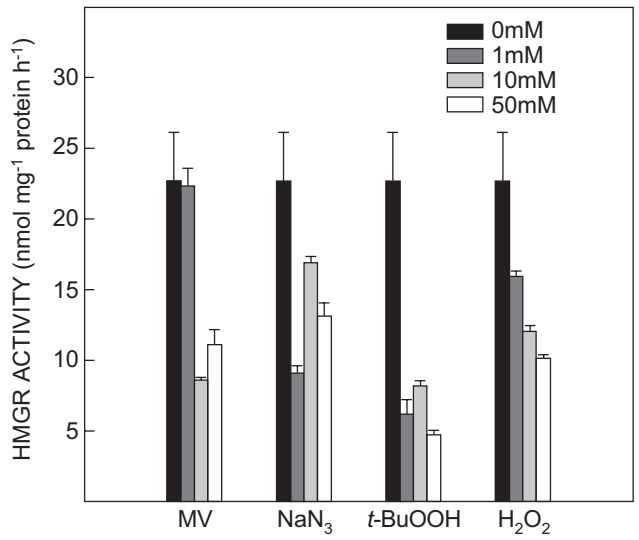

Figure 3: Effect of methyl viologen (MV), sodium azide (NaN_3), *tert*-butyl hydroperoxide (*t*-BuOOH), and hydrogen peroxide (H_2O_2) on HMGR activity in seed of 'Hass' avocado. Compounds were pulsed via the pedicel as described in Materials and Methods. Fruit (180 DAFB) were incubated at 25°C for 24h before HMGR activity was determined. Data are the mean ± SE (n = 3)

Mehdy 1994). However, and as illustrated in Figure 4a, chelation of any adventitious metal ions with EDTA resulted in a ±15% reduction in HMGR activity irrespective of the concentration of H_2O_2 in the reaction mixture. The identical trends were observed when microsomal preparations were preincubated with ROS (Figure 4b). Again, the inclusion of EDTA resulted in a ±15% reduction in HMGR activity at all concentrations of H_2O_2 tested. These findings suggested that HMGR was more sensitive to hydroperoxides than free radi-

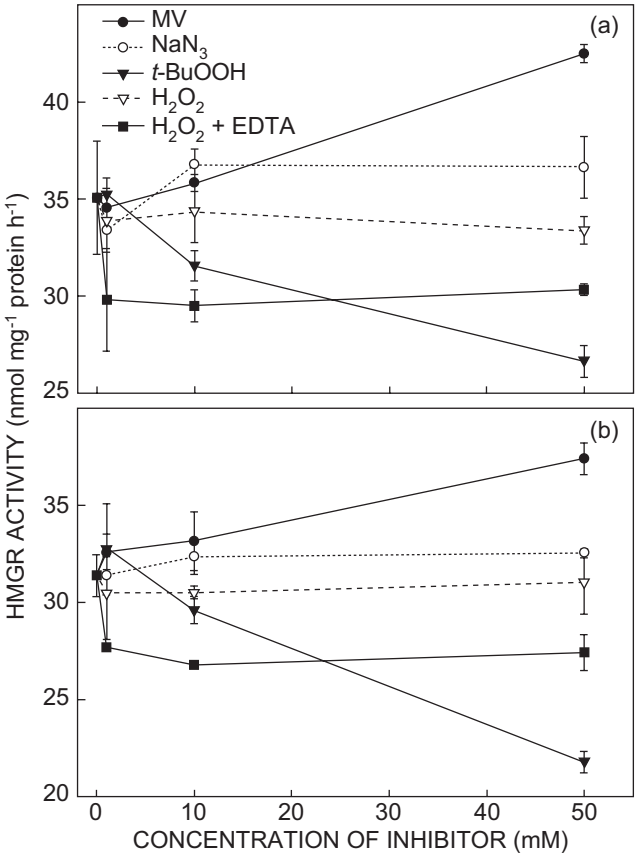

Figure 4: Effect of increasing concentrations of methyl viologen (MV), sodium azide (NaN_3), *tert*-butyl hydroperoxide (*t*-BuOOH), and hydrogen peroxide (H_2O_2) on HMGR activity in microsomal extracts of 'Hass' avocado seed. (a) Compounds were included in the assay mixture. (b) Enzyme preparations were preincubated in the presence of compounds at 30°C for 20min before HMGR activity was assayed. Data are the mean ± SE (n = 3)

cals such as the superoxide anion and hydroxyl radical. In support of this proposal, it is important to note that inhibition of HMGR activity *in vitro* by *t*-BuOOH, which is considered a model compound for lipid hydroperoxides and does not itself generate free radicals (Shi *et al*. 1993), was consistently concentration dependent (Figure 4a and b). Confirmation was obtained by studying the response of the time course of HMGR activity to increasing concentrations of *t*-BuOOH and H_2O_2 + EDTA (Figure 5). Thus, inactivation of HMGR by H_2O_2 + EDTA was rapid and complete at a concentration of 1mM (Figure 5b) whereas a similar level of inhibition by *t*-BuOOH was only achieved at 50mM (Figure 5a).

Occurrence of C7 sugars in avocado seed and effects on ROS-induced inhibition of HMGR

The sugar content and composition of 'Hass' avocado seed tissue from fruit harvested 180 DAFB is shown in Figure 6. The C7 compounds perseitol and D-*manno*-heptulose were confirmed as the major soluble sugars present. Glucose, fructose, and sucrose were also present but in much smaller amounts. Perseitol was routinely two-fold greater than

either D-*manno*-heptulose or sucrose.

Sugar alcohols such as sorbitol and mannitol have been implicated as scavengers of ROS commonly formed in response to severe stress and pathogen invasion (Stoop *et al*. 1996). Thus, it was considered that perseitol may serve a similar function in avocado fruit. To test this hypothesis the effect of exogenous perseitol and its ketose derivative, D-*manno*-heptulose, on seed HMGR activity and perseitol and D-*manno*-heptulose content was investigated. The results in Table 1 show that treatment of fruit with perseitol (1mg ml^{-1}) had little or no effect on either HMGR activity or the endogenous D-*manno*-heptulose content. However, and as expected, seed levels of perseitol increased (by 24%) following application of perseitol to detached but otherwise intact fruit. By comparison, treatment of fruit with 1.25mg ml^{-1} and 2.5D-*manno*-heptulose increased HMGR activity of seed tissue by 9% and 47% respectively. Interestingly, the D-*manno*-heptulose content of seed tissue from D-*manno*-heptulose-treated fruit declined from 13.19mg g^{-1} to 6.81mg g^{-1} dry weight whereas the concentration of perseitol increased from 52.82mg g^{-1} to 72.55mg g^{-1} dry weight. These observations suggested the possibility that perseitol may function to protect key enzymes that are required for normal growth and development, such as HMGR, from oxidative damage or ROS-induced inactivation. Support for this proposal was obtained by studying the effect of perseitol on H_2O_2-induced inhibition of HMGR activity in microsomal fractions prepared from seed tissue. As illustrated in Figure 7, addition of 30mM perseitol to the incubation medium relieved the H_2O_2-induced inhibition of HMGR at 1mM and 10mM H_2O_2 + EDTA and reduced the inhibitory effect of 50mM *t*-BuOOH from 30% to 15%.

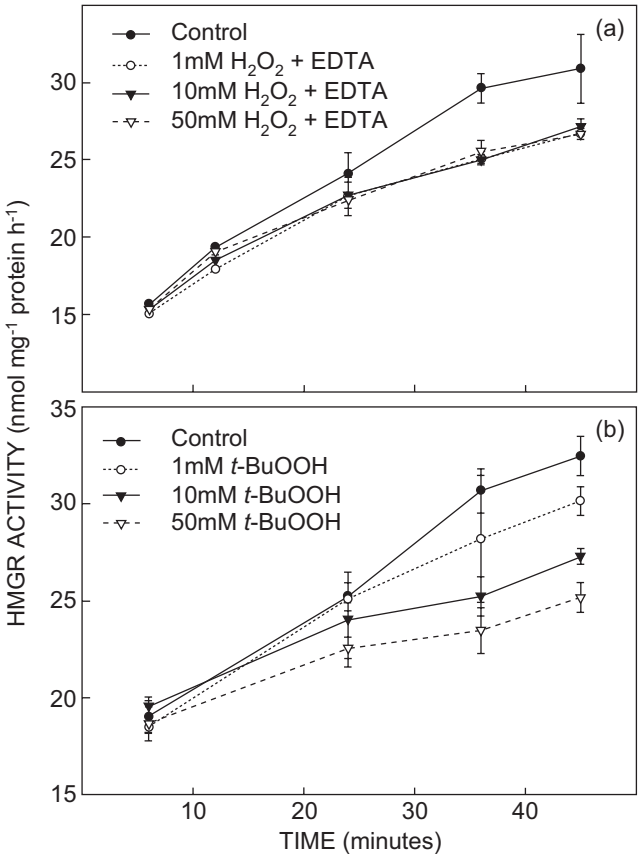

Figure 5: Kinetics of HMGR activity in microsomal preparations of 'Hass' avocado seed incubated with increasing concentrations of (a) hydrogen peroxide (H_2O_2) plus EDTA and (b) *tert*-butyl hydroperoxide (*t*-BuOOH). Assays were carried out as described in Materials and Methods and data are means ± SE (n = 3)

Discussion

D-manno-*heptulose: a regulator of carbon flux in avocado*

Although D-*manno*-heptulose and its monophosphate have been isolated from a range of plants almost nothing is known about the synthesis of D-*manno*-heptulose. In avocado D-*manno*-heptulose does not seem to be a product or precursor of perseitol, at least not in developing fruits. That the perseitol concentration was 2.5-fold higher in sap from the trunk of the tree than in sap from leaf petioles may indicate distinct sites of D-*manno*-heptulose and perseitol synthesis. It is possible that the site of D-*manno*-heptulose synthesis is the leaves and that formation occurs in a manner similar to, or coincident with the synthesis of sedoheptulose (D-*altro*-2-heptulose) particularly as amounts of D-*manno*-heptulose in plants are comparable to those of sedoheptulose (Okuda and Mori 1974). Furthermore, chemical dissection has revealed that D-*manno*-heptulose is related to perseitol and volemitol in the same manner as sedoheptulose is related to volemitol and β-sedoheptitol (Richtmyer 1970a, 1970b). A sedoheptulose ketose reductase responsible for volemitol biosynthesis from sedoheptulose has recently been suggested (Häfliger *et al*. 1999). One possible route for D-*manno*-heptulose in fruit involves: D-arabinose-5-phosphate ↔ D-ribose-5-phospate

Table 1: Effect of C7 sugars on HMGR activity and perseitol and D-*manno*-heptulose content of avocado seed tissue. Perseitol and D-*manno*-heptulose were pulsed into freshly harvested fruit (180 DAFB) via the pedicel. Fruit was incubated at 25°C for 24h before extraction and analysis of HMGR activity and quantification of endogenous perseitol and D-*manno*-heptulose by HPLC. Data are the mean ± SE (n = 3)

Treatment	HMGR activity	C7 Sugar content	
		Perseitol	D-*manno*-heptulose
	nmol mg^{-1} protein h^{-1}	mg g^{-1} dry wt	mg ml^{-1}
Control	18.79 ± 0.15	52.82 ± 0.97	13.19 ± 2.30
1.00 Perseitol	17.50 ± 1.48	65.30 ± 5.63	10.22 ± 0.99
1.25 D-*manno*-heptulose	20.52 ± 1.83	66.53 ± 5.47	8.11 ± 1.37
2.50 D-*manno*-heptulose	27.64 ± 1.18	72.55 ± 2.18	6.81 ± 3.80

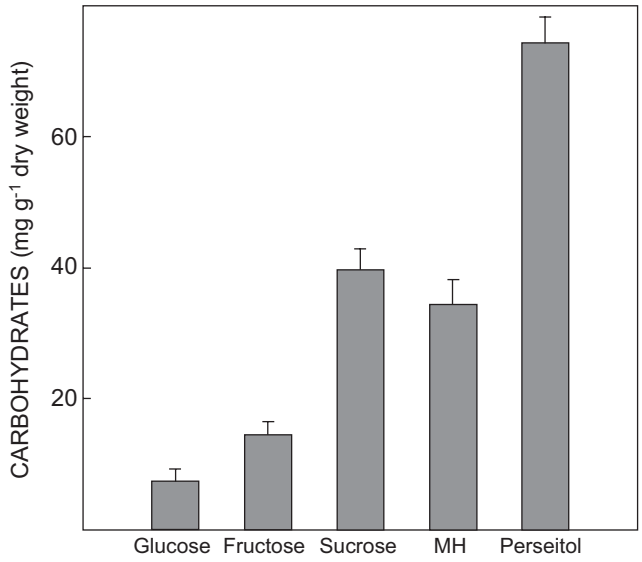

Figure 6: Sugar content and composition of 'Hass' avocado seed from fruit harvested 180 DAFB. Extracts were prepared from seed tissue and the sugars quantified by HPLC as described in Materials and Methods. Data are the mean ± SE (n = 3). MH = D-*manno*-heptulose

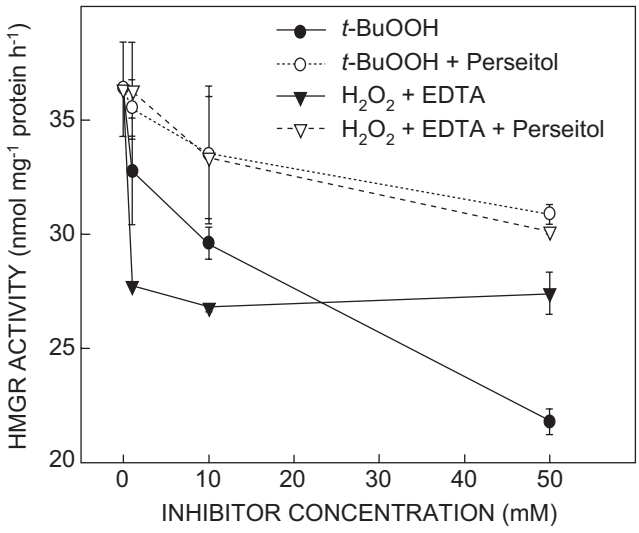

Figure 7: Effect of perseitol on *t*-BuOOH- and H_2O_2 + EDTA-induced inhibition of HMGR in microsomal preparations of 'Hass' avocado seed from fruit harvested 180 DAFB. Data are the mean ± SE (n = 3)

in which both substrates are converted to hexose 6-phosphate and triose phosphates via intermediates that include D-*manno*-heptulose 7-phosphate and D-*altro*-heptulose 1,7-*bis*phosphate (Williams *et al*. 1978). Thus, by analogy to the pathway of sedoheptulose biosynthesis, D-arabinose-5-phosphate and D-ribose 5-phosphate are the substrates for a transketolase activity and formation of D-*manno*-heptulose 7-phosphate and the 1,7- *bis*phosphate. Phosphatase activity would yield D-*manno*-heptulose a potent inhibitor of respiration that prevents entry of glucose into glycolysis (Board *et al*. 1995) and acts as a specific and competitive inhibitor of hexokinase (Leon and Sheen 2003), potentially altering the course of sugar metabolism. In avocado, these effects of D-*manno*-heptulose may be necessary for continued sugar export and phloem loading to sustain fruit growth and development. The further metabolism of D-*manno*-heptulose elsewhere in the tree may involve a keto reductase and formation of perseitol. Both D-*manno*-heptulose and perseitol appear to be products of CO_2 assimilation in avocado leaves and a recent study suggests that in this tissue, D-*manno*-heptulose is formed as a phosphorylated product of sedoheptulose-1,7 bisphosphate via condensation of dihydroxyacetone phosphate and erythrose 4-phosphate (Liu *et al*. 1999, 2002). However, these authors presented no evidence for the formation of perseitol from D-*manno*-heptulose.

Avocado fruit do not normally ripen whilst attached to the tree and remain in a mature, unripe state until harvested. It has been proposed that ripening of avocado is delayed by a 'factor' translocated from the tree or pedicel to the fruit (Biale and Young 1971, Adato and Gazit 1974). Although the identity and nature of this 'ripening inhibiting factor' remains unknown D-*manno*-heptulose is ideally suited to fulfil this role. Again, this may occur through inhibition of hexokinase and inhibition of the associated respiratory climacteric.

*On the role of D-*manno*-heptulose in avocado*

On entry to fruit by sugar, metabolism involves formation of free glucose and fructose or uridine-5'-diphosphoglucose (UDP-glucose) and fructose. The free hexoses are phosphorylated by different hexokinase isoforms to yield the corresponding hexose-6-phosphates. Depending on the energy demands of cells, the hexose-6-phosphates may enter the glycolytic pathway or be converted into UDP-glucose for synthesis of cell wall glucan. In actively growing organs it might be expected that a substantial proportion of hexose would be partitioned into cell wall glucan synthesis.

Avocado is unusual in that growth of the mesocarp by cell division and expansion continues until fruit maturity (Cowan *et al*. 1997). Furthermore, the process is dependent on a sustained supply of MVA, prenyl pyrophosphates and hence activity of HMGR. In animal cells O_2^- and H_2O_2 appear to be important factors and either abnormally high or abnormally low levels slow the rate of cell proliferation and increase apoptosis (Burdon 1996). H_2O_2 when in excess has also been shown to irreversibly inactivate animal HMGR and reduce cell division cycle activity (Omkumar and Ramasarma 1993). Furthermore, treatment of rats with D-*manno*-heptulose increases mitochondrial HMGCoA synthase activity (Quant *et al*. 1989). Since MVA is required for cell proliferation in fruit avocado growth (Cowan *et al*. 1997) mechanisms must exist to cope with excessive ROS production and the deleterious effects of ROS on MVA synthesis.

Polyols are efficient scavengers of free radicals (Jennings *et al*. 1998) and this is likely to be an additional role of C7 sugar alcohol, perseitol and its reduced form, D-*manno*-heptulose. In particular, these 7C sugars may act to protect

HMGR from ROS-mediated inactivation. H_2O_2 is thought to exert its effect on proteins indirectly through the generation of the hydroxyl radical in the presence of trace amounts of ferrous ions (Stadtman and Oliver 1991). When H_2O_2 was supplied to avocado HMGR no effect on enzyme activity was observed. However, chelation of adventitious metal ions with EDTA resulted in a ±15% reduction in HMGR activity irrespective of the concentration of H_2O_2. Inhibition of HMGR by *t*-BuOOH was consistently concentration dependent indicating that HMGR is more sensitive to hydroperoxides than free radicals such as the superoxide anion and hydroxyl radical.

In summary, D-*manno*-heptulose is a major component of the soluble sugars in avocado and could to arise by dephosphorylation of D-*manno*-heptulose 7-phosphate formed from D-arabinose-5-phosphate and D-ribose-5-phospate in a manner consistent with the biosynthesis of sedoheptulose. Both perseitol and volemitol are related alditols and together with D-*manno*-heptulose fulfill several important functions. These are: modulation of carbon flux in leaves and developing fruit to sustain the supply and partitioning of sugar to growing fruit and, as a protectant against oxidative damage of key enzymes such as HMGR required for growth and development of the fruit.

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References

Adato I, Gazit S (1974) Postharvest response of avocado fruits of different maturity to delayed ethylene treatments. *Plant Physiology* **53**: 899–902

Biale J, Young RE (1971) The avocado pear. In: Hume AC (ed) The Biochemistry of Fruits and their Products. Academic Press, London, pp 1–63

Bach TJ (1995) Some new aspects of isoprenoid biosynthesis in plants: a review. *Lipids* **30**: 191–202

Beedham C (1985) Molybdenum hydroxylases as drug-metabolizing enzymes. Drug Metabolism Reviews **16**: 119–156

Board M, Colquhoun A, Newsholme EA (1995) High K-M glucose-phosphorylating (glucokinase) activities in a range of tumor-cell lines and inhibition of rates of tumor-growth by the specific enzyme-inhibitor mannoheptulose. *Cancer Research* **55**: 3278–3285

Bueno P, Piqueras A, Kurepa J, Savouré A, Verbruggen N, Van Montagu M, Inzé D (1998) Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures. *Plant Science* **138**: 27–34

Burdon RH (1996) Control of cell proliferation by reactive oxygen species. *Biochemical Society Transactions* **24**: 1028–1032

Chappell J (1995) The biochemistry and molecular biology of isoprenoid metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**: 521–547

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* **262**: 1883–1886

Cowan AK, Cairns ALP, Bartels-Rahm B (1999) Regulation of abscisic acid metabolism: towards a metabolic basis for abscisic acid-cytokinin antagonism. *Journal of Experimental Botany* **50**: 595–603

Cowan AK, Cripps RF, Richings EW, Taylor NJ (2001) Fruit size: Towards an understanding of the metabolic control of fruit growth using avocado as a model system. *Physiologia Plantarum* **111**: 127–136

Cowan AK, Moore-Gordon CS, Bertling I, Wolstenholme BN (1997) Metabolic control of avocado fruit growth: isoprenoid growth regulators and the reaction catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Plant Physiology* **114**: 511–518

Crowell DN, Amasino RM (1991) Induction of specific mRNAs in cultured soybean cells during cytokinin or auxin starvation. *Plant Physiology* **95**: 711–715

Galichet A, Gruissem W (2003) Protein farnesylation in plants — conserved mechanisms but different targets. *Current Opinion in Plant Biology* **6**: 1–6

Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. *Plant Cell* **5**: 1439–1451

Hare PD, Cress WA, Van Staden J (1997) The involvement of cytokinins in plant responses to environmental stress. *Plant Growth Regulation* **23**: 79–103

Häfliger B, Kindhauser E, Keller F (1999) Metabolism of D-glycero-D-manno-heptitol, volemitol, in polyanthus. Discovery of a novel ketose reductase. *Plant Physiology* **119**: 191–197

Jelesko JG, Jenkins SM, Rodríguez-Concepción M, Gruissem W (1999) Regulation of tomato *HMG1* during cell proliferation and growth. *Planta* **208**: 310–318

Jennings DB, Ehrenshaft M, Pharr DM, Williamson JD (1998) Roles for mannitol and mannitol dehydrogenase in active oxygen-mediated plant defence. *Proceedings of the National Academy of Sciences USA* **95**: 15129–15133

Jones RJ, Schreiber BMN (1997) Role and function of cytokinin oxidase in plants. *Plant Growth Regulation* **23**: 123–134

Koshiba T, Saito E, Ono N, Yamomoto N, Sato M (1996) Purification and properties of flavin- and molybdenum-containing aldehyde oxidase from coleoptiles of maize. *Plant Physiology* **110**: 781–789

Leon P, Sheen J (2003) Sugar and hormone connections. *Trends in Plant Science* **8**: 110–116

Liu X, Robinson PW, Madore MA, Whitney GW, Arpaia ML, Liu X (1999) 'Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. *Journal of the American Society for Horticultural Science* **124**: 676–681

Liu X, Sievert J, Arpaia ML, Madore MA (2002) Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American Society for Horticultural Science* **127**: 108–114

Mehdy MC (1994) Active oxygen species in plant defence against pathogens. *Plant Physiology* **105**: 467–472

Mitchell DM, Cowan AK (2003) Mevastatin-induced inhibition of cell growth in avocado suspension cultures and reversal by isoprenoid compounds. *African Journal of Biotechnology* **2**: 264–270

Moore-Gordon CS, Cowan AK, Bertling I, Botha CEJ, Cross RHM (1998) Symplastic solute transport and avocado development: a decline in cytokinin/ABA ratio is related to appearance of the Hass small fruit variant. *Plant and Cell Physiology* **39**: 1027–1038

Narita JO, Gruissem W (1989) Tomato hydroxymethylglutaryl-CoA reductase is required early in fruit development but not during ripening. *Plant Cell* **1**: 181–190

Okuda T, Mori K (1974) Distribution of *manno*-heptulose and sedoheptulose in plants. *Phytochemistry* **13**: 961–964

Omkumar RV, Ramasarma T (1993) Irreversible inactivation of 3-hydroxy-3-methylgkutaryl-CoA reductase by H_2O_2. *Biochimica et Biophysica Acta* **1156**: 267–274

Quant PA, Tubbs PK, Brand MD (1989) Treatment of rats with

glucagons or mannoheptulose increases 3-hydroxy-3-methylglutaryl-CoA synthase activity and decreases succinyl-CoA content in liver. *Biochemical Journal* **262**: 159–164

Richings EW, Cripps RF, Cowan AK (2000) Factors affecting 'Hass' avocado fruit size: Carbohydrate, abscisic acid and isoprenoid metabolism in normal and phenotypically small fruit. *Physiologia Plantarum* **109**: 81–89

Richtmyer NK (1970a) The isolation of volemitol and other polyhydric alcohols from avocado seeds. *Carbohydrate Research* **12**: 135–138

Richtmyer NK (1970b) The isolation of perseitol and volemitol from Sedum, and some other observations on *Sedum* constituents. *Carbohydrate Research* **12**: 139–142

Rodríguez-Concepción M, Yalovsky S, Gruissem W (1999) Protein prenylation in plants: old friends and new targets. *Plant Molecular Biology* **39**: 865–870

Shi X, Dalal NS, Kasprzak KS (1993) Generation of free radicals from hydrogen peroxide and lipid hydroperoxides in the presence of Cr(III). Archives of Biochemistry and Biophysics **302**: 294–299

Smirnoff N, Cumbes QJ (1989) Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* **28**: 1057–1060

Stadtman ER, Oliver CN (1991) Metal-catalyzed oxidation of proteins. *Journal of Biological Chemistry* **266**: 2005–2008

Stoop JMH, Williamson JD, Pharr DM (1996) Mannitol metabolism in plants: a method for coping with stress. *Trends in Plant Science* **1**: 139–144

Tayor N, Cowan K (2001) Plant hormone homeostasis and the control of avocado fruit size. *Plant Growth Regulation* **35**: 247–255

Toyama T, Teramoto H, Takeba G, Tsuji H (1995) Cytokinin induces a rapid decrease in the levels of mRNAs for catalase, 3-hydroxy-3-methylglutaryl CoA reductase, lectin and other unidentified proteins in etiolated cotyledons of cucumber. *Plant and Cell Physiology* **36**: 1349–1359

Williamson JD, Scandalios JG (1992) Differential responses of maize catalases to abscisic acid: Vp1 transcriptional activator is not required for abscisic acid-regulated *Cat1* expression. *Proceedings of the National Academy of Sciences USA* **89**: 8842–8846

Williams JF, Blackmore PF, Clark MG (1978) New reaction sequences for the non-oxidative pentose phosphate pathway. *Biochemical Journal* **176**: 257–282

Zhu D, Scandalios JG (1994) Differential accumulation of manganese-superoxide dismutase transcripts in maize in response to abscisic acid and high osmoticum. *Plant Physiology* **106**: 173–178

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